

## A new species of *Euscorpium* (Scorpiones: Euscorpiidae) from southern Bulgaria

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**Abstract.** A new scorpion species, *Euscorpium drenskei* sp. nov., is described from the Western Rhodope Mts. in southern Bulgaria. It is characterized by an oligotrichous trichobothrial pattern, which shows a conspicuous loss of one trichobothrium in the external median patellar series ( $em = 3$ ), also observed in *E. carpathicus* (Linnaeus, 1767) and the subgenus *Alpiscorpius* Gantenbein, Fet, Largiadè & Scholl, 1999. Phylogenetic analysis of 16S rDNA marker sequences does not show any close relationship between these three groups, suggesting that the observed loss of a trichobothrium is an independent event.

**Keywords:** Scorpions, systematics, West Rhodope, 16S rDNA

The genus *Euscorpium* Thorell, 1876, widespread in southern Europe and Anatolia, is one of the most studied scorpion taxa. Despite this, the taxonomy of this genus is very complicated and still far from being resolved. This is also true for Bulgaria, where this genus has been insufficiently studied in the past. Taxonomic studies of *Euscorpium* are further hindered by the existence of cryptic species complexes, difficult to resolve even with phylogenetic analyses (Parmakelis et al. 2013, Tropea et al. 2014a). Several relatively recent studies have provided information on different *Euscorpium* populations from Bulgaria, assuming the possibility of new species (Valle 1975, Fet 2000, Teruel et al. 2004, Fet & Soleglad 2007). However, they did not focus on resolving the systematic position of these forms, but rather grouped different populations based on a few morphological characteristics, and placed them in the following species complexes: “*E. carpathicus* complex”, “*E. hadzii* complex” and “*E. mingrelicus* complex”. Most recently, Fet et al. (2014) described two new species based on molecular and morphological evidence from northern and south-western Bulgaria: *E. deltshevi* and *E. solegladi*.

Here, we describe a new species from Rhodope Mts. in southern Bulgaria, *E. drenskei* sp. nov., based on morphological and molecular evidence analyses.

### Methods and material

The trichobothrial notation follows Vachon (1974). Morphological measurements are given in millimeters (mm) following Tropea et al. (2014b). Morphological nomenclature follows Stahnke (1970), Hjel-le (1990) and Sissom (1990); the chela carinae and denticle configuration follows Soleglad & Sissom (2001); and sternum terminology follows Soleglad & Fet (2003). The map was generated by Earth Explorer 6.1, with positional and altitude data compiled through Google Maps.

All DNA work was performed in the University of Athens by PK and AP; for details on DNA extraction, amplification and sequencing, see Parmakelis et al. (2013). Phylogenetic analysis was conducted by GT as specified below. Nomenclature for reporting DNA sequences from non-type (“geneseq-3”) specimens follows Chakrabarty et al. (2013).

### Abbreviations

*V*: trichobothrial series on pedipalp chela manus ventral surface (not including  $Et_1$ ); *Pv*: trichobothria on the ventral aspect of pedipalp patella; *Pe*: trichobothria on the external surface of pedipalp patella; *et*: external terminal; *est*: external subterminal; *em*: external median; *esb*: external suprabasal; *eb<sub>a</sub>*: external basal-*a*; *eb*: external basal; *db*: dorsal basal trichobothrium on fixed finger; *Dp*: pectinal teeth number; *L*: length; *H*: height; *Lchel*: chela length; *Wchel*: chela width (= *Wchel-A* of Tropea et al. 2014a); *Lcar*: carapace length; *Wcar*: carapace width; *Lfem*: femur

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length; *Lpat*: patella length; *Lmet*: sum of the length of all metasomal segments; *Wmet*: sum of the width of all metasomal segments; *met.seg*: metasomal segment; *CarA/CarP* %: average ratio of distances from center of median eyes to anterior and posterior margins of the carapace; *DPS*: dorsal patellar spur; *DD*: distal denticle; *MD*: median denticles; *OD*: outer denticles; *ID*: inner denticles; *IAD*: inner accessory denticles; *imm.*: immature specimen (in any stage of development).

**Depositories.** GTC, personal collection of Gioele Tropea, Rome, Italy; MSNB, Museo Civico di Scienze Naturali “E. Caffi”, Bergamo, Italy; MZUR, Museo di Zoologia dell’Università di Roma “Sapienza”, Rome, Italy; NMNHS, National Museum of Natural History, Sofia, Bulgaria; VFPC: personal collection of Victor Fet, Huntington, West Virginia, USA; ZMMSU, Zoological Museum of Moscow State University, Moscow, Russia.

**Material studied.** A detailed list of material with label data is provided below.

### Phylogenetic analysis

A new 16S *rDNA* sequence for *Euscorpium drenskii* (West Rhodope Mts., Smolyan Province, Trigrad, 41.60°N, 24.38°E, 1474 m, 31 May 1999, leg. V. Fet & V. Sakalian; geneseq-3 16S) was deposited in GenBank under a submission number KP12342. Twelve published mitochondrial 16S *rDNA* sequences have been retrieved from GenBank and used for comparison: *E. tergestinus* (C.L. Koch, 1837): AJ298066; *E. avcii* Tropea, 2012: KF030937; *E. carpathicus* (Linnaeus, 1767): AY172338; *E. concinnus* (C.L. Koch, 1837): DQ989935; *E. flavicaudis* (De Geer, 1778): DQ989957; *E. germanus* (C.L. Koch, 1837): AJ249553; *E. italicus* (Herbst, 1800): DQ989956; *E. stablavskyi* Tropea, 2014: KC215605; *Euscorpium* sp.: KC215579; KC215580; KC215651; KC215644 (Gantenbein et al. 2001, Huber et al. 2001, Fet et al. 2002, Salomone et al. 2007, Parmakelis et al. 2013). The 13 sequences were aligned by eye. Phylogenetic analyses were conducted in MEGA5 (Tamura et al. 2011). All positions containing gaps and missing data were eliminated. There were a total of 366 positions in the final dataset. The phylogeny (Fig. 21) was inferred using the Neighbor-Joining algorithm (Saitou & Nei 1987); the optimal tree with the sum of branch length = 0.41174926 is shown, indicating the bootstrap values (1000 replicates) next to the clades

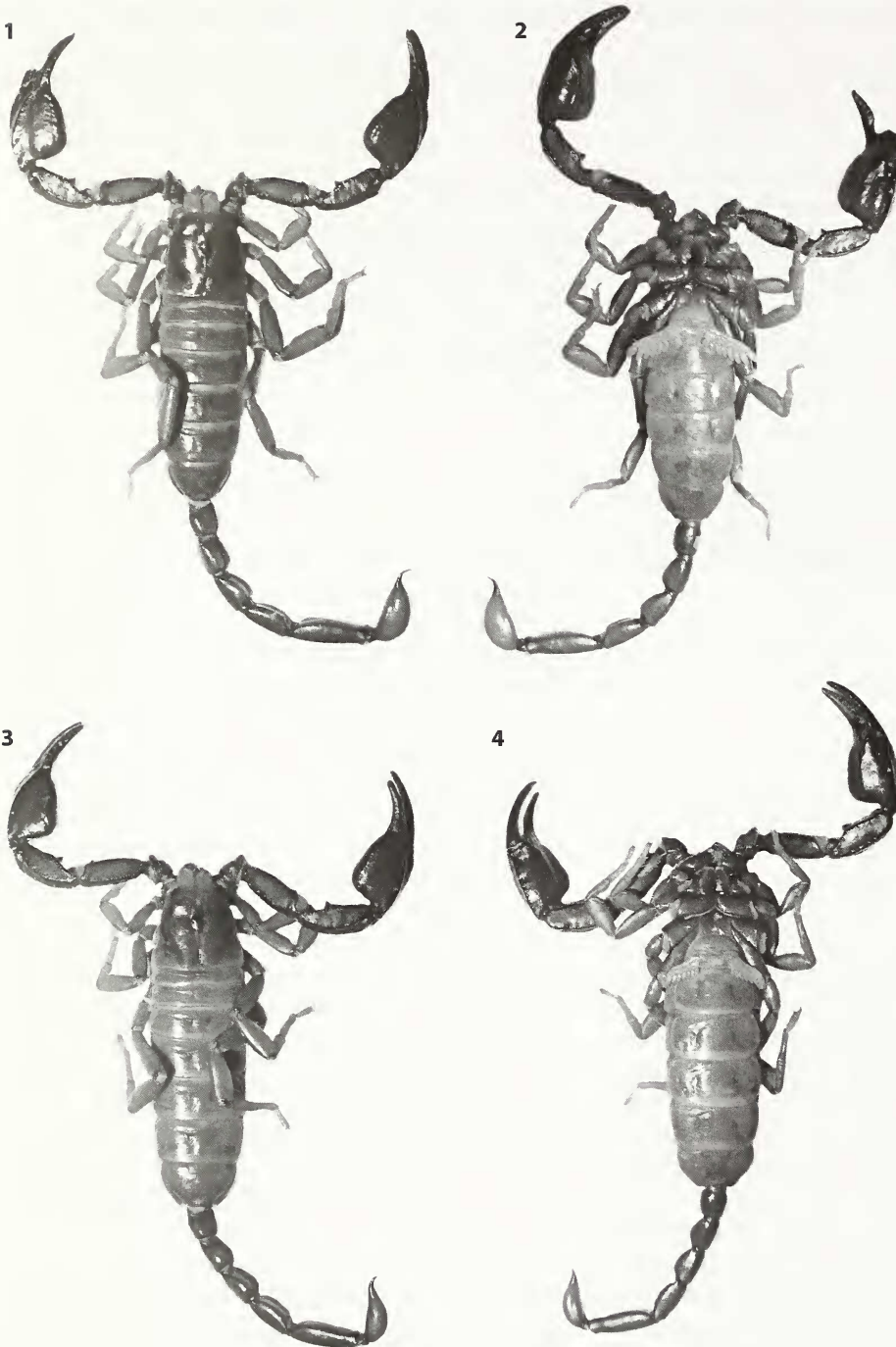
(Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as the genetic distances. The genetic distances (Tab. 2) were computed using the Kimura 2-parameter method of Kimura (1980) and are expressed as the number of base substitutions per site.

### History of study

The remote West Rhodope Mts. escaped early scorpion scholars, although the very first specimen deposited in the National Museum of Natural History, Sofia, Bulgaria (NMNHS) was collected as early as 1901 by Prince Ferdinand, the founder of this important Museum in 1889. Ferdinand I (1861–1948) of Saxe-Coburg-Gotha royalty, the Knyaz (Prince Regnant) of the independent Bulgaria since 1887, and its Tsar (King) since 1908, was an amateur lepidopterist and botanist, who promoted natural science in the Balkans.

Even though additional specimens from the West Rhodopes were collected by the most prominent Bulgarian arachnologist Pencho Drenski in 1924–1925, they have not been studied or published. The Bulgarian populations were overlooked in the most comprehensive revision of *Euscorpium* (Di Caporiaco 1950). The first data on *Euscorpium* from the West Rhodope was published by Valle (1975) who studied specimens from Smolyan Province (which currently cannot be found in the important Valle collection at Museo Civico di Scienze Naturali “Enrico Caffi”, Bergamo). Trichobothrial values given by Valle (1975) as B2 = 6 and B3 = 8 correspond to standard values (Vachon 1974) as  $eb = 4/4$  and  $eb_a = 4/4$ ; see Fet et al. (2003: 374) for a detailed scheme comparing Valle’s and Vachon’s systems of trichobothrial notation. Valle, however, did not report *em* number (D4 series) for his Smolyan specimens.

Independently, 16 specimens from the West Rhodope Mts. (now in ZMMSU) were donated to V.F. in 1984 by Dr. Christo Deltshv. This series was collected by the late Dimitar Raichev, an amateur naturalist of Chepelare, Smolyan Province, in 1981–1983. This enigmatic population was studied by V.F. and triggered his first interest in Bulgarian scorpions. Specimens were first reported as having  $em = 3$  by Fet (1993); it was clear already at that time that the Smolyan specimens do not belong to the standard Balkan “*E. mingrelicus* complex” with its et-est / est-dsb trichobothrial fixed finger ratio > 1.5; this ratio was on average only about 1.02 in the Raichev speci-



**Figs 1-2:** *Euscorpium drenskii* sp. n., male holotype  
 1. Dorsal view  
 2. Ventral view

**Figs 3-4:** *Euscorpium drenskii* sp. n., female paratype  
 3. Dorsal view  
 4. Ventral view

men series (Fet, pers. obs.). However, the species was then erroneously interpreted as *E. croaticus* (Fet 1993, Fet & Braunwalder 2000; see below for details).

Fet & Soleglad (2002) noted that an unnamed form with  $em = 3$  is found in the Rhodope Mountains of Bulgaria. Later, Fet & Soleglad (2007) provided the first comprehensive analysis of Bulgarian scorpions

on fauna, where the new species described herein was treated under “*E. carpathicus* complex”. The first DNA phylogeny from Greece and adjacent regions of the Balkans published by Parmakelis et al. (2013) indicated that *Euscorpium* fauna of the Rhodope Mountains in both Greece and Bulgaria belongs to an undescribed, basal species complex (subgenus incertae sedis).

The diverse scorpion fauna of the Rhodopes and adjacent mountain ranges is an expected feature since this region is known for high, ancient diversity of faunal elements (for detailed reviews on biogeography of many groups of vertebrates and invertebrates, see Fet & Popov 2007).

### Systematics

Genus *Euscorpis* Thorell, 1876

Subgenus incertus

*Euscorpis drenskii* Tropea, Fet, Parmakelis, Kotsakiozi & Stathi, **sp. nov.**  
(Figs 1–20, Tabs 1–2)

*Euscorpis carpathicus*: Valle 1975: 232 (in part; Bulgaria: Smolyan Province).

*Euscorpis germanus croaticus*: Fet 1993: 5 (in part; Bulgaria); Fet & Braunwalder 2000: 20 (in part; Bulgaria: Smolyan Province).

*Euscorpis carpathicus* “Group C”: Fet 2000: 55 (in part; Bulgaria: Smolyan Province); Fet & Soleglad 2002: 4.

*Euscorpis* cf. *carpathicus* “Rhodope group”: Fet & Soleglad 2007: 415, fig. 15 (in part; Bulgaria: Smolyan Province).

**Type material** (12 specimens: 6 ♂, 6 ♀)

**Holotype**: ♂, BULGARIA, West Rhodope Mts.: Smolyan Province, Shiroka Laka, 25 June 1924, leg. P. Drenski (NMHNS 275). **Paratypes**: 1 ♂, 1 ♀, West Rhodope Mts., Smolyan Province, Shiroka Laka, 25 June 1924, leg. P. Drenski (NMHNS 275); same data, 2 ♂, 1 ♀ (MZUR); same data, 1 ♂, 1 ♀ (MSNB); 3 ♀ (of which 1 imm.); West Rhodope Mts., Smolyan Province, Devin District, Trigrad, 25 June 1924, leg. P. Drenski (NMHNS 301); 1♂, West Rhodope Mts., Smolyan Province, Shiroka Laka, 26 June 1924, leg. P. Drenski (NMHNS 310).

**Other *E. drenskii* sp. nov. examined (not included in type series)**: (31 specimens: 7 ♂, 24 ♀).

BULGARIA, West Rhodope Mts.: Smolyan Province, May 1901, leg. Prince Ferdinand, 1 ♀ (NMNHS 280); Smolyan Province, Devin District, 1981–1983, leg. D. Raichev, 2 ♂, 11 ♀ (ZMMSU), Smolyan Province, Devin District, Hizha Orfei (“Orpheus Hut”), 16 June 1983, leg. D. Raichev, 1 ♂, 1 ♀ (ZMMSU);

**Tab. 1:** Measurements (mm) and morphometric ratios of *Euscorpis drenskii* sp. n.

		Holotype ♂	Paratype ♀
Total	Length	28.14	28.59
Carapace	Length	3.96	6.95
	Post. width	4.08	4.32
Metasoma	Length	11.28	10.14
Segment I	Length	1.44	1.32
	Width	1.47	1.47
Segment II	Length	1.74	1.62
	Width	1.32	1.29
Segment III	Length	1.98	1.80
	Width	1.26	1.23
Segment IV	Length	2.34	1.92
	Width	1.20	1.14
Segment V	Length	3.78	3.48
	Width	1.20	1.14
Telson	Length	3.90	3.36
Vesicle	Length	2.82	2.22
	Width	1.56	1.08
	Height	1.59	1.14
Aculeus	Length	1.08	1.14
Femur	Length	3.36	3.48
	Width	1.32	1.32
Patella	Length	3.39	3.60
	Width	1.44	1.56
Chela	Length	7.02	7.02
	Width	2.76	2.55
Movable finger	Length	4.08	3.96
Ratio	<i>CarA</i> (%)	40.91	41.72
	<i>Lcar/Lfer</i>	1.178	1.198
	<i>Lcar/Ltel</i>	1.015	1.241
	<i>Lchel/Wchel</i>	2.543	2.753
	<i>L/W met.seg I</i>	0.979	0.894
	<i>L/W met.seg II</i>	1.318	1.256
	<i>L/W met.seg III</i>	1.571	1.463
	<i>L/W met.seg IV</i>	1.950	1.684
	<i>L/W met.seg V</i>	3.150	3.052
	<i>Lmet/met.seg V</i>	2.984	2.914
	<i>Lmet/Lcar</i>	2.892	2.431
<i>Lfem/Lpat</i>	0.991	0.966	



**Figs 5–18:** *Euscorpium drenskii* sp. n. 5. Carapace. 6. External view of the chela of adult male. 7. External view of the chela of adult female. 8. Dorsal view of pedipalp patella. 9. Ventral view of pedipalp patella. 10. External view of pedipalp patella. 11. Dorsal view of pedipalp femur. 12. Ventral view of pedipalp femur. 13. Ventral view of the chela. 14. Dorsal view of the chela. 15. Telson of adult male. 16. Telson of adult female. 17. Ventral view of the metasomal segment V. 18. Lateral view of the metasomal segment V

Smolyan Province, Devin District, Yagodinska Cave, entrance, 1981, leg. D. Raichev, 1 ♂ (ZMMSU); Smolyan Province, Devin District, Yagodina, 20 May 1983, leg. P. Beron, 3 ♀ (of which 1 imm.) (NMHNS 517); Smolyan Province, Rozhen Pass, 1500 m, in moss, 23 January 1997, leg. D. Raichev, 1 ♀ (NMHNS 221); Smolyan Province, Devin District, Trigrad, Trigradski Skali Hut, 6 August 1997, leg. B. Petrov, 1 ♀ imm. (NMHNS 200); Smolyan Province, Devin District, Trigrad, 1474 m, 41.60N, 24.38E, 31 May 1999, leg. V. Fet & V. Sakalian, 3 ♂ (of which 1 imm.), 2 ♀ (of which 1 imm.) (VFPC), 1 ♂, 1 ♀ (GTC); Smolyan Province, Devin District, between Mihalkovo and Devin, 550–700 m, 1–2 September 2001, leg. B. Petrov & V. Beshkov, 2 ♀ (NMNHS 198).

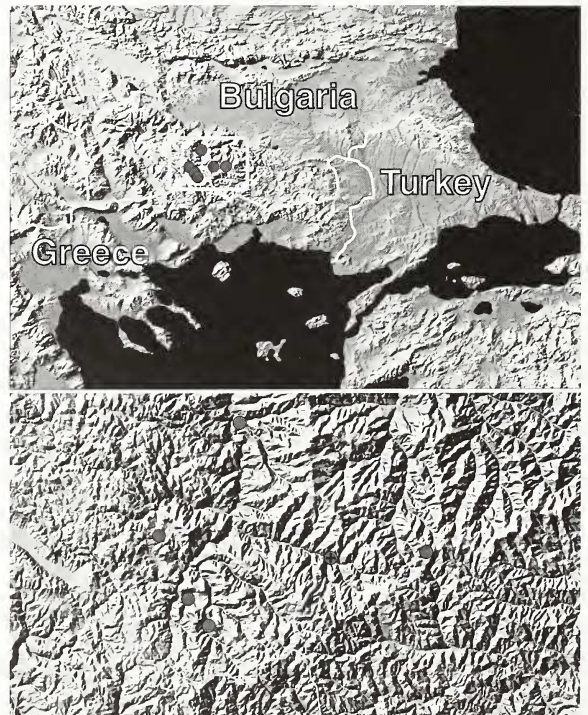


**Fig. 19:** *Euscorpium drenskii* sp. n., male holotype, ventral view of leg tarsus

**Etymology.** Named after the famous Bulgarian arachnologist Pencho Drenski (1886–1963) who collected the type specimens.

**Geographic range.** Bulgaria (south), West Rhodope Mts. (Fig. 20).

**Diagnosis.** A medium-small *Euscorpium* species, total length 28–31 mm. Colour of adults light to medium brown/reddish, carapace darker. Reticulation or marbling varies from absent to highly marked on chelicerae, carapace, mesosoma and metasoma. The number of



**Fig. 20:** Map showing type locality (+) and known distribution of *Euscorpium drenskii* sp. n.

**Table 2.** Genetic distances between 16S rDNA sequences.

The number of base substitutions per site between 13 sequences are shown. Standard error estimates are shown in the last column. See Methods and Material for explanations.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1 <i>E. sp.</i> 120F	–	0.011	0.013	0.009	0.015	0.014	0.013	0.013	0.015	0.019	0.019	0.020	0.013
2 <i>E. sp.</i> FESP9	0.037	–	0.011	0.009	0.015	0.014	0.014	0.014	0.016	0.019	0.019	0.020	0.011
3 <i>E. sp.</i> FESP21	0.055	0.043	–	0.010	0.015	0.016	0.014	0.013	0.017	0.019	0.019	0.021	0.010
4 <i>E. sp.</i> 113F	0.025	0.028	0.034	–	0.014	0.013	0.013	0.013	0.015	0.018	0.018	0.020	0.010
5 <i>E. concinnus</i>	0.081	0.085	0.091	0.075	–	0.009	0.010	0.012	0.017	0.017	0.016	0.020	0.016
6 <i>E. tergestinus</i>	0.066	0.075	0.088	0.066	0.031	–	0.009	0.012	0.015	0.018	0.016	0.019	0.015
7 <i>E. carpathicus</i>	0.060	0.069	0.075	0.060	0.034	0.031	–	0.010	0.015	0.016	0.015	0.018	0.015
8 <i>E. italicus</i>	0.060	0.072	0.072	0.063	0.051	0.045	0.039	–	0.015	0.016	0.015	0.017	0.015
9 <i>E. avcii</i>	0.075	0.087	0.096	0.078	0.099	0.081	0.078	0.078	–	0.020	0.018	0.018	0.017
10 <i>E. stablavskyi</i>	0.107	0.110	0.110	0.100	0.100	0.101	0.088	0.085	0.119	–	0.018	0.020	0.018
11 <i>E. germanus</i>	0.120	0.123	0.123	0.113	0.084	0.087	0.081	0.081	0.104	0.104	–	0.018	0.018
12 <i>E. flavicaudis</i>	0.129	0.135	0.142	0.132	0.119	0.110	0.103	0.093	0.109	0.123	0.100	–	0.021
13 <i>E. drenskii</i> sp.n.	0.055	0.043	0.037	0.034	0.094	0.084	0.078	0.081	0.090	0.103	0.119	0.142	–

trichobothria on the pedipalp manus ventral surface is 4 ( $V_{1-3} + Et$ ). The number of trichobothria on the pedipalp patella ventral surface usually is 6. The number of trichobothria on pedipalp patella external surface is:  $eb = 4$ ,  $eb_a = 4$ ,  $esb = 2$ ,  $em = 3$ ,  $est = 4$ ,  $et = 5$ . The pectinal teeth number in males usually is 8, more rarely 9; in females usually 7, more rarely 8.  $Lchel/Wchel$  ratio is 2.60 in males and 2.70 in females. Dorsal patellar spur well-developed. Femur usually more or less as long as patella;  $Lfem/Lpat$  ratio is 0.98. Carapace more or less as long as wide; average ratio  $Lcar/Wcar$  1.015 in males and 0.967 in females; average distance from center of median eyes to anterior margin of the carapace is 40.82 % of the carapace length. Average ratio of  $Lmet/Lcar$  is 2.81 in males and 2.47 in females.

#### *Trichobothrial and pectinal teeth count variation*

The variation observed in 43 studied specimens (13 ♂, 30 ♀) is given below.

*Pectinal teeth in males* (n = 13): 7/8 (1), 8/8 (5), 8/9 (2), 9/8 (3), 9/9 (2); in total, 7 in 3.85 % (1), 8 in 61.54 % (16), and 9 in 34.62 % (9); mean = 8.31, SD = 0.55.

*Pectinal teeth in females* (n = 30): ?/? (1), 6/7 (2), 7/6 (1), 7/7 (22), 7/8 (3), 8/7 (1); in total, 6 in 5.17 % (3), 7 in 87.93 % (51) and 8 in 6.90 % (4); mean = 7.02, SD = 0.35.

*Pedipalp patella trichobothria Pv* (n = 43): 6/5 (1), 6/6 (37), 6/7 (1), 7/6 (2), 6/8 (1), 7/7 (1), 8/8 (1); in total, 5 in 1.16 % (1), 6 in 89.54 % (77) %, 7 in 5.81 % (5), and 8 in 3.49 % (3); mean = 6.12, SD = 0.45.

*Pedipalp patella trichobothria Pe* (n = 43):  $et = 4/4$  (1),  $4/5$  (2),  $5/5$  (37),  $5/6$  (1),  $6/5$  (2); in total, 4 in 3.49 % (3), 5 in 93.02 % (80) and 6 in 3.49 % (3); mean = 5.00, SD = 0.27;  $em = 3/4$  (1),  $3/3$  (42); in total, 3 in 98.84 % (85) and 4 in 1.16 % (only in 1 pedipalp); mean = 3.01, SD = 0.11; in all specimens,  $est = 4/4$ ;  $esb = 2/2$ ;  $eb_a = 4/4$ ;  $eb = 4/4$ .

In addition,  $et-est / est-dsb$  ratio was measured in 16 pedipalps (of 16 different specimens): mean = 1.02, SD=0.14.

*Hemispermatothore.* Both right and left hemispermatothores of five specimens were studied. They have a well-developed lamina tapered distally; well-developed basal constriction present; truncal flexure present; median projection with primary and secondary acuminate processes, of which the secondary acuminate process is usually formed by a main tine, shaped as an elongated sickle, and from one to four secondary tines, which are more squat, and often forked with two or more tines; internal projection distally with 5–7 tines in its crown. The number and the shape of tines of the crown and of the second-

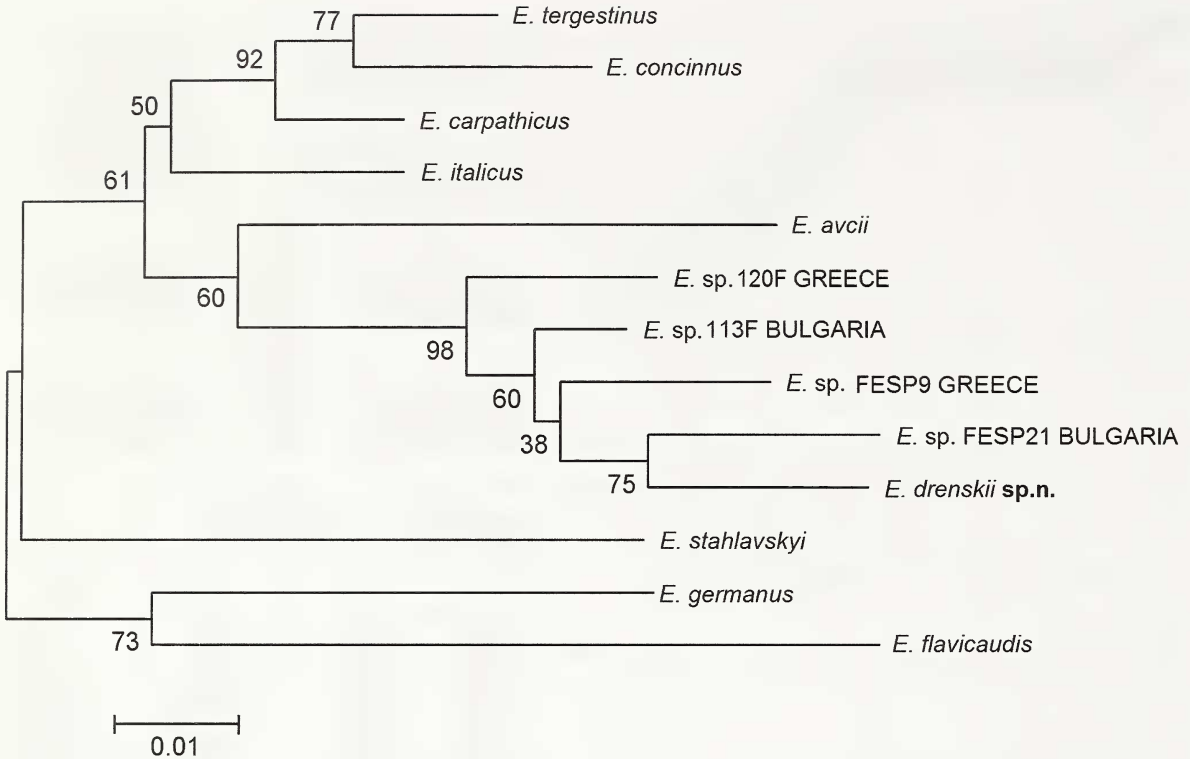


Fig. 21: A phylogenetic tree of *Euscorpilus* based on 16S rRNA mtDNA marker. See Methods and material for explanations.

ry acuminate process varied between specimens and between the right and the left hemispermatophores.

### Description of the male holotype

**Colouration:** Whole colour light brownish with carapace and pedipalps darker reddish; sternites and pectines and genital operculum very light brownish/ivory; chelicerae very light, yellowish, palms without marbling; telson yellowish, with a longitudinal lighter line and dark reddish aculeus tip; all pedipalps carinae darker, dark brown to blackish coloured; none marbling is present.

**Carapace:** A very fine granulation on whole surface is present, except in the anterior area between the anterior edge, the lateral eyes and median eyes, which is almost smooth, very finely punctated and glossy, and the lateral area behind the lateral eyes, which has a few greater granules; anterior edge granulate and more or less straight; deep and dark posterior lateral furrows; two pairs of lateral eyes (with a larger anterior eye), and a pair of median eyes, situated distally of the middle; distance from centre of median eyes to anterior margin is 40.91 % of carapace length.

**Mesosoma:** Tergites very finely granulated; sternites glossy and punctated. Small spiracles inclined about 45° downward towards outside.

**Metasoma:** Dorsal carinae on segments I–IV with spaced weakly marked granules; ventrolateral carinae absent on segment I, obsolete or smooth on segments II–IV, granulated to serrulated on segment V; ventromedian carina absent on segments I–IV, the V with spaced weakly marked granules; dorsal intercarinal spaces with a very fine granulation, smooth on the lateral and ventral surface.

**Telson:** Vesicle smooth, with ventral setae of different size, especially near the vesicle/aculeus juncture.

**Pectines:** Teeth number 8/8; middle lamellae number 6/6; several microsetae on proximal area of teeth, marginal lamellae, middle lamellae and fulcra.

**Genital operculum:** The genital operculum is formed by two longitudinally separated subtriangular sclerites; genital papillae protruding; a few microsetae are present.

**Sternum:** Pentagonal shape, type 2; more or less as long as wide, with a deep posterior emargination.

**Pedipalps:** Coxa and trochanter with tuberculated carinae. Femur: dorsal and ventral internal carinae tuberculated; dorsal external carinae formed by slightly spaced tubercles; external median carinae serrulated; ventral external carinae formed by spaced tubercles, well-formed only in the proximal one-third; anterior median formed by 13/12 spaced conical tubercles, varying in size; dorsal and ventral intercarinal spaces with granules of variable size. Patella: dorsal and ventral internal carinae tuberculated to granulated; dorsal external carinae rough; ventral external carinae from rough to granulated; dorsal intercarinal surface with a few scattered granules; ventral intercarinal surface almost smooth, only to few scattered minute granules near to ventral internal carinae is present. Dorsal patellar spur well developed. Chelal carina *D1* is distinct, strong, dark, smooth to rough; *D4* is rounded and rough; *V1* is distinct, strong, dark and rough with a few serrulated tubercles proximally; *V3* rounded, dark, smooth to rough; external carina granulated; intercarinal tegument from smooth to rough with granules of variable size. Typical *Euscorpium* chela finger dentition.

**Trichobothria:** Chela: trichobothria on the pedipalp manus ventral surface 4/4 ( $V_{1-3} + Et_1$ ). Patella ventral (*Pv*): 6/6. Patella external (*Pe*): *et* = 5/5, *est* = 4/4, *em* = 3/3, *esb* = 2/2, *eb<sub>a</sub>* = 4/4, *eb* = 4/4. Femur: trichobothrium *d* is slightly proximal to *i*, while trichobothrium *e* is distal to both *d* and *i*, and situated on dorsal external carina.

**Legs:** With two pedal spurs; no tarsal spur; ventral row of tarsus III with a total of 8/6 worn-out spinules, of increasing size from proximal to distal, ending with a decentralized spinule. Granulation well present on dorsal and ventral surface of leg femora, it is mostly marked and dark ventrally.

**Chelicerae:** Movable finger: the dorsal distal denticle is much smaller than the ventral distal denticle; ventral edge is smooth with brush-like setae on the inner part; dorsal edge has five denticles: one large distal, two small subdistal, one large median, and a small basal. Fixed finger has four denticles: one distal, one subdistal, one median, and one basal, the last two in a fork arrangement; the internal surface has brush-like setae.

## Discussion

The species of the genus *Euscorpium* in Bulgaria have been insufficiently studied. Limited information was given mostly in relatively recent papers (Valle 1975,

Fet 2000, Teruel et al. 2004, Fet & Soleglad 2007). Some authors assumed the possibility of new species present in Bulgaria (Teruel et al. 2004, Fet & Soleglad 2007); however, they did not focus on resolving the systematic position of these forms, but rather grouped several populations based on their morphology, and addressed them as belonging to species groups or complexes: “*E. carpathicus* complex”, “*E. hadzii* complex” and “*E. mingrelicus* complex”.

Recently, two new Bulgarian species were described: a widespread *Euscorpium deltshevi* Fet, Graham, Webber & Blagoev, 2014 (a form of “*E. carpathicus* complex”), from the Stara Planina (= Balkan) Mts. in central Bulgaria; and a more localized *E. solegladi* Fet, Graham, Webber & Blagoev, 2014 (a form of “*E. hadzii* complex”), from south-western Bulgaria. Both of these species belong to the subgenus *Euscorpium* s.str.

In addition, Parmakelis et al. (2013), in a large phylogenetic study of *Euscorpium* from Greece and adjacent countries, included two other populations from the south-western Bulgaria, which are not closely related to two species described by Fet et al. (2014), but instead group with several populations from northeastern Greece (clade E4 in Parmakelis et al. 2013). In our current opinion, these closely related populations belong to several good species which our team is currently describing (Tropea et al. in prep.).

The new species described in this paper, *E. drenskii*, has not been included in the study of Parmakelis et al. (2013). However, we used 16S rDNA to construct a phylogenetic tree, which places this species in a clade outside of the subgenus *Euscorpium* s.str., together with the neighbouring populations from southwestern Bulgaria and northeastern Greece (clade E4 in Parmakelis et al. 2013). This confirms that *E. drenskii*, *E. carpathicus* (type species of the subgenus *Euscorpium* s.str.) and the subgenus *Alpiscorpium* are three distinct and strongly supported clades with a long history of independent evolution, despite of the peculiar reduced trichobothrial series *em* = 3.

According to our preliminary phylogeny constructed based on 16S rDNA data, *E. drenskii*, together with other populations from southwestern Bulgaria and northeastern Greece form a larger clade, with *Euscorpium avicii* as its closest clade. This clade is well-separated from the subgenus *Polytrichobothrius* Birula, 1917 (type species *E. italicus*) as well as from the subgenus *Euscorpium* Thorell, 1876 s.str. (here



represented by *E. carpathicus*, *E. tergestinus*, and *E. concinnus*).

*E. drenskii* exhibits genetic distance of 3.4 % to 5.5 % from other populations of its clade (clade E4 in Parmakelis et al. 2013), which is equal or higher than among other closely related species (e.g., *E. carpathicus* has a genetic distance of 3.4 % and 3.1 % from *E. tergestinus* and *E. concinnus*, respectively), and 7.8 % to 14.2 % from the remaining species of our phylogenetic tree. Note the large genetic divergence shown between *E. drenskii* and *E. carpathicus* (type species of the subgenus *Euscorpis*), which is 7.8 %, and with *E. germanus* (type species of the subgenus *Alpiscorpius*), which is as high as 11.9 %. It is clear that the new species does *not* belong to the subgenus *Euscorpis* s.str., and that the shared condition of  $em = 3$  between these three groups is homoplasious.

Regarding its trichobothrial pattern, *E. drenskii* is one of the most oligotrichous species in the entire genus *Euscorpis*; in fact, only a few species of the subgenus *Alpiscorpius* have a lower summary number of patellar trichobothria ( $Pv + Pe$ ) (e.g. *E. germanus*, *E. alpha* and *E. gamma*). So far, no species has been described with such low values outside of the subgenus *Alpiscorpius* (or related to it). With  $Pv = 6$  and  $Pe = 22$  ( $et = 5$  and  $em = 3$ ), *E. drenskii* has the same trichobothrial values as *E. mingrelicus* s.str. and *E. croaticus* Di Caporiacco 1950, and an even lower value than *E. mingrelicus ciliciensis* Birula 1898 ( $Pv = 7$  and  $Pe = 22$ ). It should be also be noted that, among the populations phylogenetically close to *E. drenskii*, none have  $em = 3$ , and most have  $Pv = 6-9$  and  $Pe = 23-25$  ( $et = 5-7$  and  $em = 4$ ) (Tropea et al. in prep.). Thus this character state is probably independently derived (autapomorphic). A very similar situation is presented by *E. carpathicus* in south-western Romania, which has  $em = 3$ , while phylogenetically close *E. deltsbevi* from Serbia and northern Bulgaria has  $em = 4$  (Fet et al. 2014, unpublished data of Tropea).

With its trichobothrial pattern, which should be considered the most clear diagnostic character set for *E. drenskii*, it can be easily distinguished from most of the other *Euscorpis* species. In fact, as explained above, only *E. carpathicus*, *E. mingrelicus*, and *E. croaticus* have exactly the same trichobothrial pattern as *E. drenskii*. However, *E. drenskii* can be quite readily differentiated from these forms as follows:

From *E. carpathicus*, *E. drenskii* is distinguished mainly by: (1) the number of  $Pv = 6$  in *E. drenskii* versus normally 8 in *E. carpathicus*; (2) *E. drenskii* has

$Pe-et = 5$  versus usually 6 and 7 in *E. carpathicus*. In addition, *E. carpathicus* has a dark brown colour, and inhabits south-western Romania.

From *E. mingrelicus*, *E. drenskii* can be easily distinguished by the ratio of distances between trichobothria on fixed finger,  $et-est / est-dsb$ , which is  $> 1.5$  in *E. mingrelicus* complex (Bonacina 1980), while it is just over 1 in *E. drenskii*. In addition, *E. mingrelicus* has a dark brown colour.

The last species, which has the same number of trichobothria as *E. drenskii*, is *E. croaticus*. This form has recently been elevated to the status of species by Graham et al. (2012), and, according to their phylogenetic tree based on COI data, it clustered with the subgenus *Alpiscorpius*. However, due to its ambiguous morphological features, *E. croaticus* has not been assigned to any subgenus (for more information see Graham et al. 2012). Fet (1993) identified specimens of *E. drenskii* from Trigrad, Bulgaria, as *E. croaticus*. However, while the latter groups with the subgenus *Alpiscorpius*, in our phylogeny *E. drenskii* forms a clade strongly separated from *Alpiscorpius*. Morphologically, these two species can be distinguished by (1) a different number of pectinal teeth, 8–9 in males and 7 in females of *E. drenskii*, versus 6–7 (usually 7) in males and 5–6 (usually 6) in females of *E. croaticus* (Tropea, unpublished data); (2) a slightly shorter metasoma in proportion to the carapace in *E. drenskii*; on average 2.81 (maximum ratio 2.89) in *E. drenskii* versus 3.01 in the lectotype of *E. croaticus*; (3) a more slender metasoma in *E. drenskii*;  $Lmet/Wmet$  on average 1.75 (lowest value 1.74) in *E. drenskii*, compared to 1.66 in the lectotype of *E. croaticus*. In addition, *E. croaticus* is found only in northwestern Croatia (Di Caporiacco 1950, Bonacina 1980, Graham et al. 2012).

## Conclusions

In the past, the genus *Euscorpis* has been intensively studied; over 40 species and subspecies were described. Most of these taxa were later downgraded to subspecies status or moved to synonymy. However, since 1999, when this genus had only 4 recognized species, the number steadily increased and has gradually reached 17 in 2007. Thanks to further detailed studies, based both on morphological and molecular data, from 2012 to the present, the species number now increased to 43 (including *E. drenskii*), and several other species are in press or in description. This large increase in species diversity, and in the studies

that led to establishing these taxa, reflect a great degree of speciation and endemism in *Euscorpium*, which are often restricted to very limited areas such as a mountain range or an island, or a small group of mountains or islands.

Another interesting point that was understood during these studies, and noted for the first time by Tropea (2013), is that the existing subgeneric division of the genus *Euscorpium* was not consistent with the taxonomic situation. Parmakelis et al. (2013), in a much larger and detailed molecular phylogenetic study, arrived at the same result. Currently, there are a number of forms without a clear subgeneric placement. These include the new species described herein, *E. drenskii*. According to a traditional identification key, it is a part of the subgenus *Euscorpium*, but genetically it is completely separate, and could belong to a separate subgenus (or even genus); therefore we addressed it here as a “subgenus incertus”.

Further studies, resulting in improved identification keys, are needed to bring order in this growing and complicated scorpion group. This goal could be supported by a study of hemispermaphores, which was quite decisive, e.g., in the recent revisions of scorpion genera *Iurus* Thorell, 1876 and *Protoiurus* Soleglad, Fet, Kovařík & Yağmur, 2012 (Iuridae) (Kovařík et al. 2010, Soleglad et al. 2012). Using hemispermaphores is not an easy or universal criterion, as they are only present in males, which are usually represented in collections by fewer number than the females. In addition, to analyse these organs, the specimens must be dissected, and a high variability between specimens and even between the left and right hemispermaphore is present in *Euscorpium* (Tropea pers. obs.). Thus, to obtain a reliable result, a large number of adult males should be dissected. It must be pointed out, however, that in *E. drenskii* these organs, although variable, show a more complex secondary acuminate process than in many other *Euscorpium*, but are nevertheless similar to other Balkan populations related to *E. drenskii* (Tropea in prep.).

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#### References

- Bonacina A 1980 Sistematica specifica e sottospecifica del complesso “*Euscorpium germanus*” (Scorpiones, Chactidae). – *Rivista del Museo Civico di Scienze Naturali “Enrico Caffi”* (Bergamo) 2: 47-100
- Chakrabarty P, Warren M, Page LM & Baldwin CC 2013 GenSeq: An updated nomenclature and ranking for genetic sequences from type and non-type sources. – *ZooKeys* 346: 29-41 – doi: 10.3897/zookeys.346.5753
- Di Caporiacco L 1950 Le specie e sottospecie del genere “*Euscorpium*” viventi in Italia ed in alcune zone confinanti. – *Memorie /Atti della Accademia Nazionale dei Lincei*, serie VIII, vol. II, sez. III, fasc. 4: 159-230
- Felsenstein J 1985 Confidence limits on phylogenies: an approach using the bootstrap. – *Evolution* 39: 783-791 – doi: 10.2307/2408678
- Fet V 1993 Notes on *Euscorpium mingrelicus* (Kessler, 1874) from the Caucasus. – *Rivista del Museo Civico di Scienze Naturali “Enrico Caffi”* (Bergamo) 16: 1-8
- Fet V 2000 Scorpions (Arachnida, Scorpiones) from the Balkan Peninsula in the collections of the National Museum of Natural History, Sofia. – *Historia Naturalis Bulgariae* 11: 47-60
- Fet V & Braunwalder ME 2000. The scorpions (Arachnida, Scorpiones) of the Aegean area: current problems in taxonomy and biogeography. – *Belgian Journal of Zoology* 130 (Suppl. 1): 17-22
- Fet V, Gantenbein B, Fet EV & Popa V 2002. *Euscorpium carpathicus* (Linnaeus, 1767) from Romania (Scorpiones: Euscorpidae): mitochondrial DNA data. – *Biogeographica* (Paris) 78(4): 141-147
- Fet V, Graham MR, Webber MM & Blagoev G 2014 Two new species of *Euscorpium* (Scorpiones: Euscorpidae) from Bulgaria, Serbia, and Greece. – *Zootaxa* 3894: 83-105 – doi: 10.11646/zootaxa.3894.1.7

- Fet V & Popov A (eds) 2007 Biogeography and ecology of Bulgaria. Monographiae Biologicae 82. Springer, Dordrecht–Boston. 687 pp. – doi: 10.1007/978-1-4020-5781-6
- Fet V & Soleglad ME 2002 Morphology analysis supports presence of more than one species in the “*Euscorpium carpathicus*” complex (Scorpiones: Euscorpiidae). – *Euscorpium* 3: 1–50
- Fet V & Soleglad ME 2007 Fauna and zoogeography of scorpions (Arachnida: Scorpiones) in Bulgaria. – In: Fet V & Popov A (eds) Biogeography and ecology of Bulgaria. Monographiae Biologicae 82. Springer, Dordrecht–Boston. pp. 405–422 – doi: 10.1007/978-1-4020-5781-6\_12
- Fet V, Soleglad ME, Gantenbein B, Vignoli V, Salomone N, Fet EV & Schembri PJ 2003 New molecular and morphological data on the “*Euscorpium carpathicus*” species complex (Scorpiones: Euscorpiidae) from Italy, Malta, and Greece justify the elevation of *E. c. sicanus* (C. L. Koch, 1837) to the species level. – *Revue suisse de Zoologie* 110: 355–379
- Gantenbein B, Soleglad ME & Fet V 2001 *Euscorpium balearicum* Caporiacco, 1950, stat. nov. (Scorpiones: Euscorpiidae): molecular (allozymes and mtDNA) and morphological evidence for an endemic Balearic Islands species. – *Organisms, Diversity & Evolution* 1: 301–320 – doi: 10.1078/1439-6092-00027
- Graham MR, Webber MM, Blagoev G, Ivanova N & Fet V 2012 Molecular and morphological evidence supports the elevation of *Euscorpium germanus croaticus* Di Caporiacco, 1950 (Scorpiones: Euscorpiidae) to *E. croaticus* stat. nov., a rare species from Croatia. – *Revista Ibérica de Aracnología* 21: 41–50
- Hjelle JT 1990 Anatomy and morphology. In: Polis GA (ed) *Biology of scorpions*. Stanford University Press, Stanford, CA. pp. 9–63
- Huber D, Gantenbein B, Fet V & Scherabon B 2001 *Euscorpium carpathicus* (L.) from Austria (Scorpiones: Euscorpiidae): phylogenetic position clarified by mitochondrial DNA analysis. In: Fet V & Selden PA (eds) *Scorpions 2001*. In Memoriam Gary A. Polis. British Arachnological Society, Burnham Beeches, Bucks. pp. 273–278
- Kimura M 1980 A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. – *Journal of Molecular Evolution* 16: 111–120 – doi: 10.1007/BF01731581
- Kovařík F, Fet V, Soleglad ME & Yağmur EA 2010 Etudes on iurids, III. Revision of the genus *Iurus* Thorell, 1876 (Scorpiones: Iuridae), with a description of two new species from Turkey. – *Euscorpium* 95: 1–212
- Parmakelis A, Kotsakiozi P, Stathi I, Poulikarakou S & Fet V 2013 Hidden diversity of *Euscorpium* (Scorpiones: Euscorpiidae) in Greece revealed by multilocus species-delimitation approaches. – *Biological Journal of the Linnean Society* 110: 728–748 – doi: 10.1111/bij.12170
- Saitou N & Nei M 1987 The neighbor-joining method: a new method for reconstructing phylogenetic trees. – *Molecular Biology and Evolution* 4: 406–425
- Salomone N, Vignoli V, Frati F & Bernini F 2007 Species boundaries and phylogeography of the “*Euscorpium carpathicus* complex” (Scorpiones: Euscorpiidae) in Italy. – *Molecular Phylogenetics and Evolution* 43: 502–514 – doi: 10.1016/j.ympev.2006.08.023
- Sissom WD 1990 Systematics, biogeography and paleontology. In: Polis GA (ed.) *Biology of scorpions*. Stanford University Press, Stanford, CA. pp. 64–160
- Soleglad ME & Fet V 2003 The scorpion sternum: structure and phylogeny (Scorpiones: Orthosterni). – *Euscorpium* 5: 1–33
- Soleglad ME, Fet V, Kovařík F & Yağmur EA 2012 Etudes on Iurids, V. Further revision of *Iurus* Thorell, 1876 (Scorpiones: Iuridae), with a description of a new genus and two new species. – *Euscorpium* 143: 1–170
- Soleglad ME & Sissom WD 2001 Phylogeny of the family Euscorpiidae Laurie, 1896: a major revision. In: Fet V & Selden PA (eds.) *Scorpions 2001*. In Memoriam Gary A. Polis. British Arachnological Society, Burnham Beeches, Bucks, UK. pp. 25–112
- Stahnke HL 1971 Scorpion nomenclature and mensuration. – *Entomological News* 81: 297–316
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M & Kumar S 2011 MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. – *Molecular Biology and Evolution* 28: 2731–2739 – doi: 10.1093/molbev/msr121
- Teruel R, Fet V & Armas L de 2004 A note on the scorpions from the Pirin Mountains, southwestern Bulgaria (Scorpiones: Buthidae, Euscorpiidae). – *Euscorpium* 14: 1–11
- Tropea G 2013 Reconsideration of the taxonomy of *Euscorpium tergestinus* (Scorpiones: Euscorpiidae). – *Euscorpium* 162: 1–23
- Tropea G, Parmakelis A, Sziszkosz N, Balanika K & Boudierka A 2014a A new species of *Euscorpium* Thorell, 1876 from Naples Province, Italy (Scorpiones: Euscorpiidae). – *Euscorpium* 182: 1–12
- Tropea G, Yağmur EA & Yeşilyurt F 2014b A new species of *Euscorpium* Thorell, 1876 (Scorpiones, Euscorpiidae) from the Antalya Province, Southern Turkey. – *Euscorpium* 184: 1–13
- Vachon M 1974 Etude des caractères utilisés pour classer les familles et les genres de scorpions (Arachnides). 1. La trichobothriotaxie en aracnologie. Sigles trichobothriotaxie et types de trichobothriotaxie chez les scorpions. – *Bulletin du Muséum National d’Histoire naturelle, Paris* 140: 859–958
- Valle A 1975 Considerazioni intorno alle sottospecie di *Euscorpium carpathicus* (L.) (Scorpiones, Chactidae). – *Ateneo Parmense, Acta Naturalia* 11: 209–234